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	First Named Inventor	Huang, Shu-Gui
	Application Number	10/669,382
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	Group Art Unit	1651
	Examiner Name	Fernandez, Susan Emily
	Title: *High-Throughput Turbidometric Assay for Screening inhibitors of protein disulfide isomerase activity*	

Sir:

I, Shu-Gui Huang, do hereby declare as follows:

I am the Director of Lead Discovery at AGY Therapeutics, Inc., and I am a co-inventor of the above captioned patent application. I have worked in the field of biochemistry, particularly as it relates to pharmaceutical research and development. Following completion of my doctoral studies, I have worked for more than 19 years as a research associate, research scientist, group leader and scientific director. In my present position, I oversee the drug discovery process for treatment of central nervous system diseases; this involves sophisticated compound screening, ADME toxicology screening, assay development, secondary assays and high-throughput screening.

I have read and understood the Office Action of March 3, 2006, and the references cited therein, particularly with respect to the rejection of the claims as being unpatentable over Bonfils *et al.*, (Eur. J. Biochem., 1998, 254:420-427) in view of Qvist *et al.* (U.S. Patent no. 6,110,689), Cahoon *et al.* (WO 00/22100) and Moussebois *et al.* (U.S. Patent no. 4,397,960), Ryser (WO94/04185); and Dunlay et al. (U.S. Patent no. 5,989,835).

In my opinion, I believe that the combination of the methods and adaptation to high-throughput screening is not obvious for the following reasons.

The key to the success of the claimed assay is an accurate mixing step, which has to be brief and thorough, and a clear stop to the reaction. If the mixing is not brief, one will miss the initial reaction phase. If the mixing is not thorough, the reaction kinetics can show a lag

because the reactants did not "meet" each other instantly after mixing, thereby confounding the resulting values obtained. And without a distinct stop, which does not alter the readout of the assay, it is difficult to perform a high throughput assay, with multiple parallel reactions. The prior art does not teach one of skill in the art how to reasonable perform such an assay.

The method described by Bonfils *et al.* was for a cuvette assay. The turbidometric assay described in the reference is extremely difficult to perform accurately, even in a cuvette, one reaction at a time, performed by a well-skilled experimenter. It is particularly difficult to obtain accurate results using these techniques in a parallel assay format.

The adaptation to 384-well assay is even more challenging, because one has to ensure that the reaction starts, continues, and ends simultaneously in all 384 wells in the plate. The examples of the instant application performed mixing by a liquid handling system, which has 384 pipettes. The wells are small and required substantial testing of automation parameters (e.g. speed, volume, position). If the pipeting speed was too low, mixing was not sufficient, if it is too high, solutions will splash out of wells. The reaction volume must be kept low, because of cost considerations relating to high throughput screening campaigns. The exact positioning of the pipettes with respect to the wells is also crucial for rapid and thorough mixing without causing splashes of the reactants.

Therefore it is not straightforward, nor is it obvious to adapt a conventional cuvette assay to a parallel well plate assay due to: the nature of a kinetics assay where brief and thorough mixing is essential, the restrictions placed on high throughput screening campaigns; and the requirement for an accurate stop reagent. Prior to the teachings of the present invention and the modifications required to generate the functional assay under HTS conditions, it was unclear whether a high through put assay could be achieved.

It is also not obvious which inhibitor or stop reagent to use for the PDI assay. Hydrogen peroxide could disrupt the aggregated insulin chain due to oxidation of the SH groups back to the oxidized form and thus change the OD reading. In addition, hydrogen peroxide is an aggressive oxidant and could react with the enzyme and substrates. Whether this stop reagent would work or not required specific experimental demonstration and inventive input, and could not have been expected based on the teachings of the prior art.

In conclusion I feel that the difficulties that had to be overcome in order to create the presently claimed high throughput assay should be given weight. As an scientist that frequently

converts low throughput assays to the high throughput assays, I wish to emphasize that this assay required more than routine experimentation in order to obtain a workable system.

I hereby declare that all statements made herein of my own knowledge are true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted,

Date: 05/20/2005

By _____
Shu-Gui Huang